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## PPARα agonist-induced rodent tumors: Mode(s) of action and human relevance

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## PPARα agonist-induced rodent tumors: Mode(s) of action and human relevance

1995: ILSI-sponsored workshop

"Do peroxisome proliferative compounds pose a hepatocarcinogenic hazard to humans?"

*Regul. Toxicol. Pharmacol. 27:47-60, 1998*

1995 goal: Consensus regarding interpretation of data relative to assessment of human risk.

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## 1995: ILSI-sponsored workshop consensus

•PPARα agonists are non-genotoxic carcinogens

•Enhanced cell proliferation is critical for PPARα agonist-induced hepatocarcinogenesis

•Oxidative stress unlikely to have major role, may contribute

•Marked species differences: rodents sensitive, humans insensitive

•Peroxisome proliferation & enhanced cell proliferation good interspecies markers for PPARα agonist induced hepatocarcinogenesis

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## 1995: ILSI-sponsored workshop consensus

•Peroxisome proliferation in the rodent is a consequence of PPARα activation

•PPARα activation is involved in PPARα agonist-induced liver growth

•Differences in PPARα levels and activity between rodents and humans

•The interspecies differences in PPARα are consistent with lack of gene induction in human hepatocytes

•Enzyme induction (e.g. ACO) and cell proliferation are important adjuncts in the characterization of dose-response curve for PPARα agonist-induced liver tumors

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## 1995: ILSI-sponsored workshop conclusion

"……it is unlikely that peroxisome proliferators are carcinogenic to humans under anticipated conditions and levels of exposure, although their carcinogenic potential cannot be ruled out under extreme conditions of exposure. Risk assessment should be done on a case-by-case basis, using a margin-of-exposure approach. Furthermore, risk assessment approaches should utilize weight of the evidence and incorporate consideration of all available data."

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## 2001-02: ILSI-sponsored working group

Update the 1998 report, given new information obtained on the mechanism(s) by which PPARα agonists produce certain carcinogenic responses in rodents, and advances in the understanding of the underlying genetic factors that mediate biochemical and cellular responses induced by PPARα agonists.

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## 2001-02: ILSI-sponsored working group

### Peroxisome Proliferation (PPARα Agonist) Panel

- **J. Klaunig, Indiana Univ (Chair)**
- **M. Babich, U.S. CPSC**
- **K. Baetcke, U.S. EPA**
- **R. Brown, U.S. FDA/CDRH**
- **J. Cook, Pfizer, Inc.**
- **C. Corton, Consultant**
- **M. Creek, Valent**
- **R. David, Eastman Kodak**
- **J. DeLuca, Merck**
- **J. El Hage,U.S. FDA/CDER**
- **D. Lai, U.S. EPA**
- **R. McKee, Exxon/Mobil**
- **J. Peters, Penn State Univ**
- **J. Popp, Purdue Pharma**
- **R. Roberts, Aventis Pharma**
- **J. Swenberg, Univ of North Carolina**
- **A. Tobia, Bayer Crop Sciences**

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## 2001-02: ILSI-sponsored working group

Review process

Reassess current understanding of PPARα agonist-induced carcinogenesis MOA

Identify key events for MOA, decide whether causal or associative, indicate strength of evidence to support MOA, and specificity for rodent-induced tumors

Determine if PPARα agonist-induced rodent tumors should be considered relevant and applicable in human cancer hazard/risk assessments of substances belonging to this group of chemicals; using case studies

Analyze available data to describe the modes of action by which Leydig cell and pancreatic acinar cell tumors are produced in rats by PPARα agonists that also produce liver tumors (*i.e.*, an exploration of the "Tumor Triad")

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## 2001-02: ILSI-sponsored working group

Relevant "new" information

•PPARα required for PPARα agonist-induced cell proliferation and liver cancer (null mouse data; partially included in report but not discussed at 1995 workshop)

•Additional reports demonstrating low PPARα mRNA in human liver

•Identification of truncated mutants, mutant PPARα in humans

•Evidence from potent PPARα agonists, and stably transfected cell lines confirming species difference in vitro

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## 2001-02: ILSI-sponsored working group

Relevant "new" information

•Evidence of polymorphic PPREs in PPARα target gene (ACO)

•Differences in trans-activation of PPARα agonists between human and murine PPARα

•Non-human data demonstrating the lack of increased markers of peroxisome proliferation or cell proliferation in response to PPARα agonists

•In vivo human data showing no induction of ACO in response to fibrates

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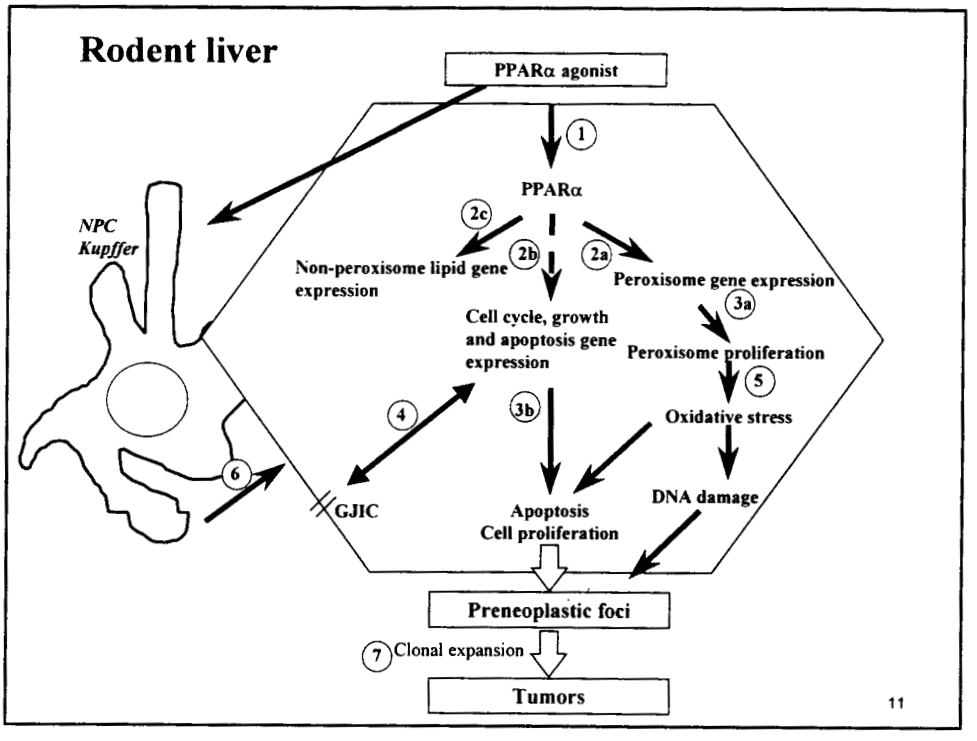


## Significant differences in species response

In Vitro: Hepatocytes/liver cell lines treated with PPARα agonists

| Cells | Induction of ACO mRNA/ACO activity |
|---|---|
| Rodent hepatocytes | ++ |
| Rodent hepatoma (Fao) | ++ |
| Human hepatocytes | +/- |
| Human hepatoma* | +/- |

*Stably transfected HepG2 cells expressing significantly increased levels of human PPARα are still refractory to increases in ACO induced by PPARα agonists.

*Human liver cells exhibit significantly reduced change in a standard biomarker of PPARα activation*

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## Significant differences in species response

In Vivo: Liver response after treatment with PPARα agonists

| Species | ACO mRNA/ACO activity | Cell proliferation* |
|---|---|---|
| Rats, mice | ++ | ++ |
| Hamster | + | + |
| Guinea pig | +/- | +/- |
| Non-human primate | +/- | - |

*As determined by measuring relative liver weight or increased BrdU incorporation.

*While rats, mice and hamsters exhibit increases in a biomarker of PPARα activation (ACO) and changes associated with causative factors linked to PPARα agonist-induced liver tumors, guinea pigs and non-human primates are refractory to these events.*

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## Significant differences in species response

In Vivo: Liver response after treatment with PPARα agonists

•Liver biopsies from human patients treated with fibrates do not exhibit marked peroxisome proliferation

•The mRNA encoding ACO is not increased in liver from human patients treated with fibrates

•Expression of PPARα is relatively low in human liver, hepatocytes and liver cell lines

•Human clinical evidence does not show a correlation between increased liver tumors and treatment with PPARα agonists

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# Framework

**Relationship**

- **Causal**: Required step for PPARα MOA, based on empirical evidence
- **Associative** : Events that are occurring but may or may not be causally linked to the MOA

**Weight of Evidence**

- **Strong:** several studies which support that MOA, preferably with multiple PPARα agonists from multiple laboratories: limited evidence of contradiction.
- **Weak:** normally defined by having a single study with a single PPARα agonist from a single laboratory or a significant amount of contradiction in the literature

**Specificity to PPARα-induced rodent hepatic tumors**

- **High** is defined as unique to this PPARα **MOA.**
- **Low** is defined as not unique to PPARα **MOA**

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**Key Events for Mode of Action (MOA) in Rodent Liver Carcinogenesis and Preceding Events**

| | Event | Relationship | Weight of evidence | Specificity |
|---|---|---|---|---|
| 1. | Activation of PPARα | Causal | Strong | High |
| 2a. | Peroxisome gene expression | Associative | Strong | High |
| 2b. | Cell cycle, growth and apoptosis gene expression | Associative | Weak | Low |
| 2c. | Non-peroxisome lipid gene expression | Associative | Strong | Low |
| 3a. | Peroxisome proliferation | Associative | Strong | High |
| 3b. | Perturbation of cell proliferation and apoptosis | Causal | Strong | Low |
| 4. | Inhibition of GJIC | Associative | Strong | Low |
| 5. | Hepatocyte oxidative stress | Associative | Weak | Low |
| 6. | Kupffer cell-mediated events | Associative | Strong | Low |
| 7. | Selective clonal expansion | Causal | Strong | Low |

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| PPARα Agonist MOA Key Events |
|---|
| **Activation to Active Metabolite** |
| **Activation of PPARα** |
| Peroxisome Gene Expression |
| Cell Cycle Gene Expression |
| Lipid Gene Expression |
| Peroxisome Proliferation |
| **Perturbation of Cell Growth** |
| Inhibition of GJIC |
| Hepatocyte oxidative stress |
| Kupffer cell mediated events |
| **Selective clonal expansion** |

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## Case Studies

Two case studies (chemicals) involving PPAR α interaction/ modulation have been chosen to illustrate the application of the Enhanced Mode of Action Framework

- These studies addressed only liver tumors

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## Basis for selection of model chemicals

1. A rich animal database, limited human data:
   - Diethylhexyl phthalate (DEHP)
2. A less robust animal database, but human data:
   - Clofibrate

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## Di- (2-ethylhexyl)phthalate (DEHP)

- Plasticizer used in medical devices and consumer products formed from PVC
- Induces liver tumors in rats and mice
- Induces peroxisome proliferation in rodent livers
- Not mutagenic

*Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

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| **DEHP: PPARα MOA Key Event** | **Evidence in Rodents** | |
|---|---|---|
| **Activation to Active Species** | Yes | seen *in vivo* and *in vitro* |
| **Activation of PPARα** | Yes | Concentration-related activation *in vitro*, no downstream events in PPARα-null mice *in vivo* |
| Gene Expression: Peroxisome | Yes | *In vivo* increases in mRNA in wild-type versus no increase in PPARα-null mice |
| Gene Expression: Cell Cycle | Unknown | |
| Gene Expression: Lipid | Yes | Increase in gene expression for fatty acid metabolism enzymes |
| Peroxisome Proliferation | Yes | Dose-related increases in peroxisomal enzymes |
| **Perturbation of Cell Growth** | Yes | cell replication *in vivo, in vitro,* |
| Inhibition of GJIC | Yes | GJIC inhibited *in vivo, in vitro* |
| Hepatocyte oxidative stress | Yes/No | Conflicting data *in vivo*, increased $H_2O_2$ levels *in vitro* |
| Kupffer cell mediated events | Yes | Kupffer cell-mediated cell proliferation altered *in vitro* |
| **Selective clonal expansion** | Yes | DEHP promotes initiated cells *in vivo* |

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## DEHP

*Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

*Yes*

*Are the key events in the rodent mode of action for DEHP plausible in humans?*

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| DEHP: MOA Key Event | Evidence in Rodents | Evidence in Humans (Primates) |
|---|---|---|
| **Activation to Active Metabolite** | Yes | Yes |
| **Activation of PPARα** | Yes | Yes |
| Gene Expression: Peroxisome | Yes | Unknown |
| Gene Expression: Cell Cycle | Unknown | Unknown |
| Gene Expression: Lipid | Yes | Unknown |
| Peroxisome Proliferation | Yes | No |
| **Perturbation of Cell Growth** | Yes | No |
| Inhibition of GJIC | Yes | No |
| Hepatocyte oxidative stress | Yes/No | Unknown |
| Kupffer cell mediated events | Yes | Unknown |
| **Selective clonal expansion** | Yes | Unknown |

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## DEHP

*Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

*Yes*

*Are the key events in the rodent mode of action for DEHP plausible in humans?*

*Yes, although unlikely*

*Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?*

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| DEHP: MOA Key Event | Evidence in Rodents | Evidence for Key event in humans (primates) | Evidence in humans, taking into account similarities & differences in kinetics and dynamics |
|---|---|---|---|
| Activation to Active Metabolite | Yes | Yes | Yes |
| Activation of PPARα | Yes | Yes | Yes |
| Gene Expression: Peroxisome | Yes | Unknown | Not likely |
| Gene Expression: Cell Cycle | Unknown | Unknown | Unknown |
| Gene Expression: Lipid | Yes | Unknown | Unknown |
| Peroxisome Proliferation | Yes | No | No/Not likely |
| Perturbation of Cell Growth | Yes | No | No/Not likely |
| Inhibition of GJIC | Yes | No | No |
| Hepatocyte oxidative stress | Yes/No | Unknown | Unknown |
| Kupffer cell mediated events | Yes | Unknown | Unknown |
| Selective clonal expansion | Yes | Unknown | Unknown |

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## Conclusions: DEHP

*1. Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

*Yes*

*2. Are the key events in the rodent mode of action for DEHP plausible in humans?*

*Yes, although unlikely*

*3. Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?*

*No, unlikely*

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## Conclusions: DEHP

1. In rodents, liver tumor induction by DEHP follows the PPARα MOA.
2. In humans, a key causal event-perturbation of cell proliferation does not occur. Additionally, associative events-peroxisome proliferation and inhibition of GJIC do not occur.
3. CONCLUSION: The rodent MOA for PPARα agonist-induced liver cancer is unlikely to occur in humans following DEHP exposure.

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## Clofibrate

- Therapeutic agent used to treat hyperlipidemia in humans
- Induces liver tumors in rats
- Induces peroxisome proliferation in rodent liver
- Not mutagenic

*Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

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| Clofibrate: PPARα MOA Key Events | Evidence in Rodents |
|---|---|
| **Activation to Active Metabolite** | Yes |
| **Activation of PPARα** | Yes |
| Peroxisome Gene Expression | Yes |
| Cell Cycle Gene Expression | Unknown |
| Lipid Gene Expression | Yes |
| Peroxisome Proliferation | Yes |
| **Perturbation of Cell Growth** | Yes |
| Inhibition of GJIC | Yes |
| Hepatocyte oxidative stress | Yes |
| Kupffer cell mediated events | Unknown |
| **Selective clonal expansion** | Yes |

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## Clofibrate

*Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

*Yes*

*Are the key events in the rodent mode of action for clofibrate plausible in humans?*

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| **Clofibrate: PPARα MOA Key Events** | **Evidence in Rodents** | **Evidence in Humans (Primates)** |
|---|---|---|
| **Activation to Active Metabolite** | Yes | Yes |
| **Activation of PPARα** | Yes | Yes/Unknown |
| Peroxisome Gene Expression | Yes | No |
| Gene Expression: Cell Cycle | Unknown | Unknown |
| Gene Expression: Lipid | Yes | Yes – Indirect |
| Peroxisome Proliferation | Yes | No |
| **Perturbation of Cell Growth** | Yes | No |
| Inhibition of GJIC | Yes | No |
| Hepatocyte oxidative stress | Yes | Unknown |
| Kupffer cell mediated events | Unknown | Unknown |
| **Selective clonal expansion** | Yes | Unknown |

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## Clofibrate

*Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

*Yes*

*Are the key events in the rodent mode of action for clofibrate plausible in humans?*

*Yes, although unlikely*

*Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?*

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| Clofibrate : **MOA Key Event** | Evidence in Rodents | Evidence for Key event in humans (primates) | Evidence in humans , taking into account similarities & differences in kinetics and dynamics |
|---|---|---|---|
| **Activation to Active Metabolite** | Yes | Yes | Yes |
| **Activation of PPARα** | Yes | Yes | Yes |
| Gene Expression: Peroxisome | Yes | Unknown | Unknown |
| Gene Expression: Cell Cycle | Unknown | Unknown | Unknown |
| Gene Expression: Lipid | Yes | Unknown | Unknown |
| Peroxisome Proliferation | Yes | No | No |
| **Perturbation of Cell Growth** | Yes | No | No |
| Inhibition of GJIC | Yes | No | No |
| Hepatocyte oxidative stress | Yes/No | Unknown | Unknown |
| Kupffer cell mediated events | Yes | Unknown | Unknown |
| **Selective clonal expansion** | Yes | Unknown | Unknown |

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## Conclusions: Clofibrate

*1. Is the weight of evidence sufficient to establish the PPARα-agonist mode of action for hepatic neoplasia in rodents?*

*Yes*

*2. Are the key events in the rodent mode of action for DEHP plausible in humans?*

*Yes, although unlikely*

*3. Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?*

*No, unlikely*

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## Conclusions: Clofibrate

1. In rodents, liver tumor induction by clofibrate follows the PPARα MOA.

2. In humans, a key causal event-perturbation of cell proliferation does not occur. Additionally, associative events-peroxisome proliferation and inhibition of GJIC do not occur

3. CONCLUSION: The rodent MOA for PPARα agonist-induced liver cancer is unlikely to occur in humans following clofibrate exposure.

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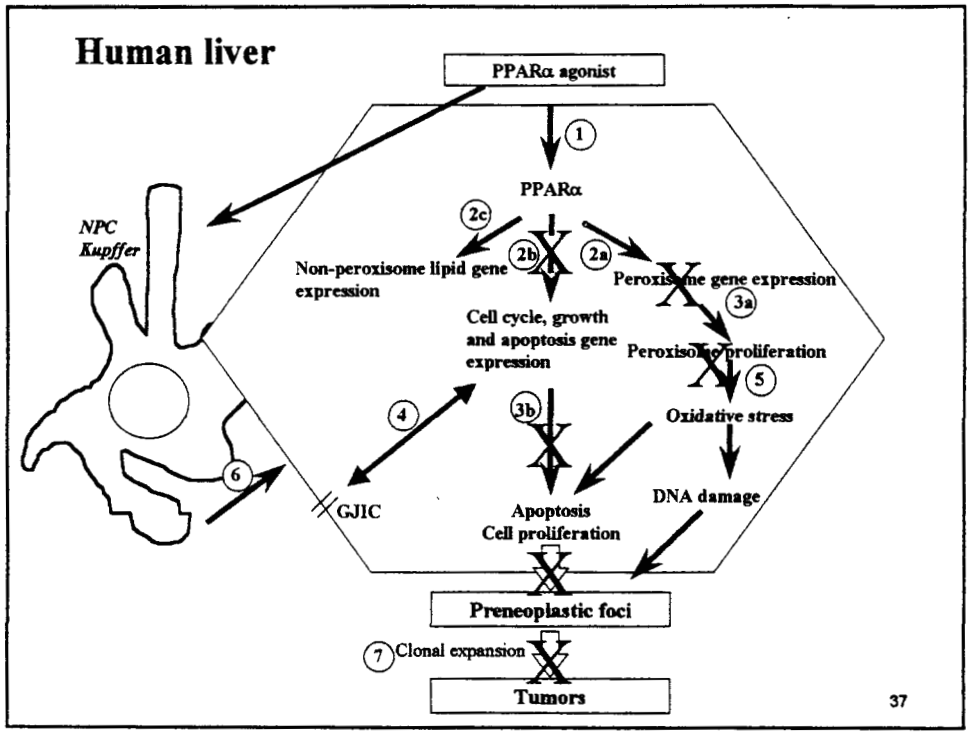
| **Rat/mouse MOA key events for liver tumors** | **Is this key event in the animal MOA plausible in humans?** | **Taking into account kinetic and dynamic factors, is this key event in the animal MOA plausible in humans?** |
|---|---|---|
| 1. Activation of PPARa | YES | YES |
| 2a. Expression of peroxisomal genes | Not likely | Not likely |
| 2b. PPARa -mediated expression of cell cycle, growth and apoptosis | Unknown | Unknown |
| 2c. Non-peroxisome lipid gene expression | YES - this is the molecular basis of human therapeutic response to hypolipidemic drugs | YES |
| 3a. Peroxisome proliferation | Not likely | Not likely – no or weak response in human biopsy material and in non-human primates |
| 3bi. Perturbation of cell proliferation | Not likely- not seen in many independent studies of human hepatocytes *in vitro*; not measured in humans *in vivo*; not seen in non-human primates *in vivo* or *in vitro*; not seen in hamsters | Not likely |
| 3bii. Perturbation of apoptosis | Not likely-not seen in limited studies of human hepatocytes *in vitro*; not measured in humans *in vivo* | Not likely |
| 4. Inhibition of GJIC | Not likely- no inhibition in primates *in vitro* or *in vivo* or in human hepatoctyes *in vitro* | Not likely |
| 5. Hepatocyte oxidative stress | Unknown | Unknown |
| 6. Kupffer cell mediated events | Unknown | Unknown |
| 7. Selective clonal expansion | Unknown – no response seen in non-human primates | Unknown |
| 8. Liver tumors | Not likely | Not likely |


Human liver
PPARα agonist
1
PPARα
2c
2b
2a
NPC
Kupffer
Non-peroxisome lipid gene expression
Peroxisome gene expression
3a
Cell cycle, growth and apoptosis gene expression
Peroxisome proliferation
5
4
3b
Oxidative stress
6
GJIC
Apoptosis
Cell proliferation
DNA damage
Preneoplastic foci
7 Clonal expansion
Tumors
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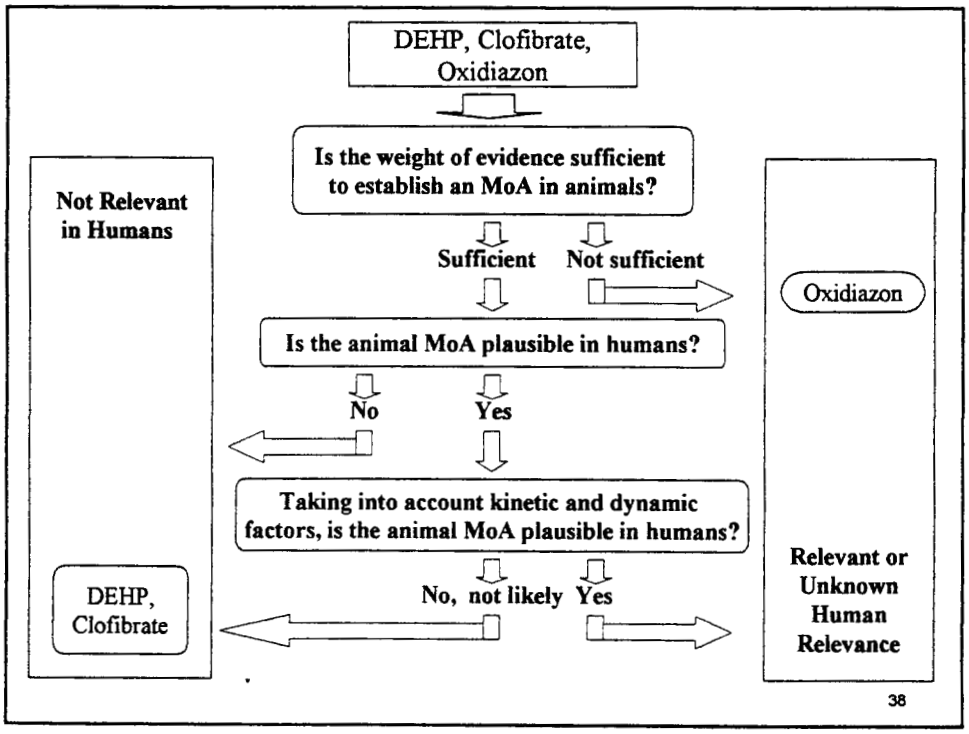
DEHP, Clofibrate, Oxidiazon
Is the weight of evidence sufficient to establish an MoA in animals?
Not Relevant in Humans
Sufficient
Not sufficient
Oxidiazon
Is the animal MoA plausible in humans?
No
Yes
Taking into account kinetic and dynamic factors, is the animal MoA plausible in humans?
DEHP, Clofibrate
No, not likely
Yes
Relevant or Unknown Human Relevance
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## Leydig cell tumors

- Hypothesized that these tumors arise via peroxisome proliferation
- PPARα agonists induce LCTs in rats by two potential pathways
  - enhancement of growth factor expression within the testis (Pathway 1)
  - inhibition of testosterone biosynthesis (Pathway 2)

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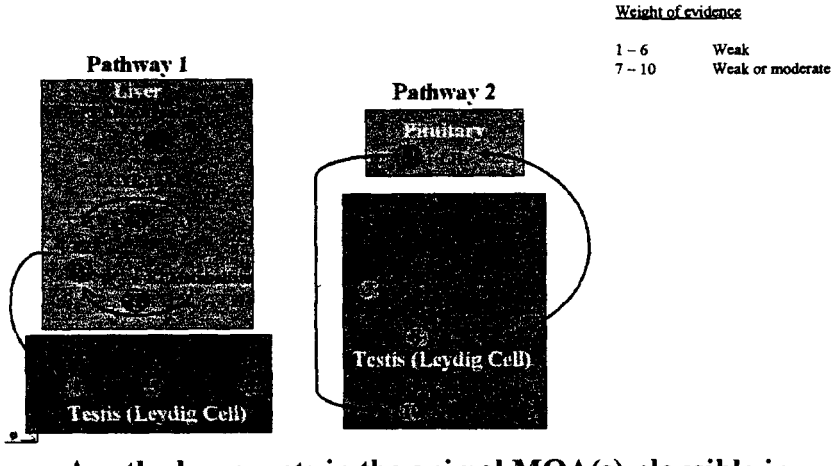
## Leydig cell tumors




**Are the key events in the animal MOA(s) plausible in humans?**

- Hard to evaluate until rodent key events are clearer.

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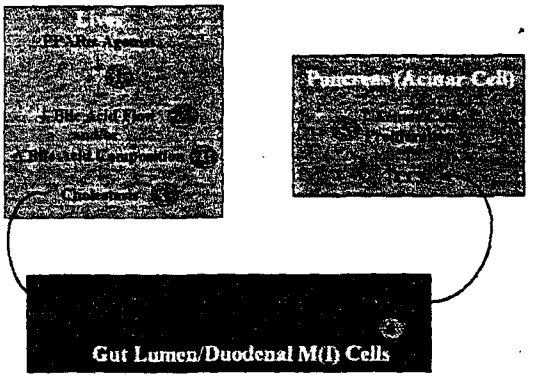
## Pancreatic acinar cell tumors




Weight of evidence

| | |
|---|---|
| 1 | Moderate |
| 2a, b | Moderate |
| 3, 4 | Weak |
| 5 | Strong *but nonspecific* |

1. PPARα/RXRα reduces HNF-4 binding to the DR-1 sequence which increases the transcription of cholesterol 7α-hydroxylase, the rate limiting step of bile acid synthesis

**Are the key events in the animal MOA(s) plausible in humans?**

- Hard to evaluate until rodent key events are clearer.

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## Key Points

**LIVER**

A plausible MOA for PPARα agonist-induced liver tumors with substantial weight of evidence for key events

Substantial weight of evidence to show that this MOA is unlikely to operate in humans

**LCTs**

A plausible MOA for PPARα agonist-induced Leydig cell tumors with some evidence for key events

Some evidence that this MOA is unlikely to operate in humans

**PACTs**

A plausible MOA for PPARα agonist-induced pancreatic acinar cell tumors with some evidence for key events

Some evidence that this MOA is unlikely to operate in humans

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## Data Gaps

**Liver**

- ACO is only a marker gene of peroxisome proliferation
- Identity of genes regulated by PPARα to regulate apoptosis and proliferation?
- Can then determine species differences in the genes responsible for tumors

**LCTs and PACTs**

- Link between PPARα and first events – some of the evidence is circumstantial
- additional data required
- Are these tumors secondary to liver changes?

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## Acknowledgements

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- J. Klaunig, Indiana Univ (Chair)
- M. Babich, U.S. CPSC
- K. Baetcke, U.S. EPA
- R. Brown, U.S. FDA/CDRH
- J. Cook, Pfizer, Inc.
- C. Corton, Consultant
- M. Creek, Valent
- R. David, Eastman Kodak
- J. DeLuca, Merck
- J. El Hage,U.S. FDA/CDER
- D. Lai, U.S. EPA
- R. McKee, Exxon/Mobil
- J. Peters, Penn State Univ
- J. Popp, Purdue Pharma
- R. Roberts, Aventis Pharma
- J. Swenberg, Univ of North Carolina
- A. Tobia, Bayer Crop Sciences

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